Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells.

Journal: Nature

Publication Year: 2011

Authors: W A Pastor, U J Pape, Y Huang, H R Henderson, R Lister, M Ko, E M McLoughlin, Y Brudno, S Mahapatra, P Kapranov, M Tahiliani, G Q Daley, X S Liu, J R Ecker, P M Milos, S Agarwal, A Rao

PubMed link: 21552279

Funding Grants: Generation of regulatory T cells by reprogramming, Training in the Biology of Human Embryonic Stem Cells and Emerging Technologies II

Public Summary:
5-hydroxymethylcytosine (5hmC) is a modified base present at low levels in diverse cell types in mammals. 5hmC is generated by the TET family of Fe(II) and 2-oxoglutarate-dependent enzymes through oxidation of 5-methylcytosine (5mC). 5hmC and TET proteins have been implicated in stem cell biology and cancer, but information on the genome-wide distribution of 5hmC is limited. Here we describe two novel and specific approaches to profile the genomic localization of 5hmC. The first approach, termed GLIB (glucosylation, periodate oxidation, biotinylation) uses a combination of enzymatic and chemical steps to isolate DNA fragments containing as few as a single 5hmC. The second approach involves conversion of 5hmC to cytosine 5-methylenesulphonate (CMS) by treatment of genomic DNA with sodium bisulphite, followed by immunoprecipitation of CMS-containing DNA with a specific antiserum to CMS. High-throughput sequencing of 5hmC-containing DNA from mouse embryonic stem (ES) cells showed strong enrichment within exons and near transcriptional start sites. 5hmC was especially enriched at the start sites of genes whose promoters bear dual histone 3 lysine 27 trimethylation (H3K27me3) and histone 3 lysine 4 trimethylation (H3K4me3) marks. Our results indicate that 5hmC has a probable role in transcriptional regulation, and suggest a model in which 5hmC contributes to the 'poised' chromatin signature found at developmentally-regulated genes in ES cells.

Scientific Abstract:
5-hydroxymethylcytosine (5hmC) is a modified base present at low levels in diverse cell types in mammals. 5hmC is generated by the TET family of Fe(II) and 2-oxoglutarate-dependent enzymes through oxidation of 5-methylcytosine (5mC). 5hmC and TET proteins have been implicated in stem cell biology and cancer, but information on the genome-wide distribution of 5hmC is limited. Here we describe two novel and specific approaches to profile the genomic localization of 5hmC. The first approach, termed GLIB (glucosylation, periodate oxidation, biotinylation) uses a combination of enzymatic and chemical steps to isolate DNA fragments containing as few as a single 5hmC. The second approach involves conversion of 5hmC to cytosine 5-methylenesulphonate (CMS) by treatment of genomic DNA with sodium bisulphite, followed by immunoprecipitation of CMS-containing DNA with a specific antiserum to CMS. High-throughput sequencing of 5hmC-containing DNA from mouse embryonic stem (ES) cells showed strong enrichment within exons and near transcriptional start sites. 5hmC was especially enriched at the start sites of genes whose promoters bear dual histone 3 lysine 27 trimethylation (H3K27me3) and histone 3 lysine 4 trimethylation (H3K4me3) marks. Our results indicate that 5hmC has a probable role in transcriptional regulation, and suggest a model in which 5hmC contributes to the 'poised' chromatin signature found at developmentally-regulated genes in ES cells.